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Ecdysteroid levels/profiles of the parasitoid wasp, *Diapetimorpha introita*, reared on its host, *Spodoptera frugiperda* and on an artificial diet

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Abstract

Diapetimorpha introita is an ichneumonid ectoparasitoid of the fall armyworm, *Spodoptera frugiperda*. Since it has been reported that *D. introita* wasps reared on an artificial diet exhibit a significantly lower percentage of adult eclosion and fecundity than host-reared wasps, this study was undertaken to elucidate the factors responsible for the reduced viability observed in diet-reared wasps. A system of markers has been devised to track the development (from the initiation of cocooning through adult eclosion) of *D. introita.* Although wasps reared on artificial diet developed more slowly than did those reared on host pupae, both diet- and host-reared wasps passed through the same stages of development — the eyes enlarged and moved backward, the gut was purged and upon ecdysis the exarate pupa emerged. The thorax was the first to darken, followed by the head and then the abdomen. Pharate pupal formation occurred before gut purge. Two peaks of hemolymph ecdysteroids were observed, one in wasps in which gut purge was almost complete and the second in day-2 exarate pupae. Ecdysone and 20-hydroxyecdysone were the major ecdysteroids present in hemolymph sampled at these times. Small quantities of 20,26-dihydroxyecdysone, polar ecdysteroids and/or possibly 26-hydroxyecdysone were also present. In six stages of development, hemolymph ecdysteroid titers were significantly higher in host-reared than in diet-reared wasps (Eye 1, Eye 2, Gut Purge 2, Pharate Pupa, Head/Thorax Dark, and Abdomen Dark). Relatively high percentages of mortality were observed in diet-reared wasps in four of these stages and in two others which occurred in close proximity to one of the stages, the Abdomen Dark stage. Thus, insufficient ecdysteroid in the hemolymph may be responsible, in part, for the relatively high percentage of mortality that occurred in wasps reared on an artificial diet. Published by Elsevier Science Ltd.

Keywords: Diapetimorpha introita; Parasitoid; Developmental markers; Ecdysteroids; Molting; Metamorphosis; Artificial rearing

1. Introduction

The value of using insect parasitoids and predators as the biological control component of IPM programs is well known (Knipling 1980, 1992; Gross and Pair, 1986; Grenier et al., 1994). Mass rearing and augmentative releases of such parasitoid wasps as *Encarsia formosa* (for control of *Trialeurodes vaporariorum*), *Trichogramma* sp. (for control of *Heliothis* sp., *Ostrinia nubilalis*, *Mamestra brassicae* and others), *Catolaccus grandis* (for control of boll weevil) and *Edovum puttleri* (for control of *Leptinotarsa decemlineata)* have been used successfully in greenhouses in the field or in field trials (van Lentern and Woets, 1988; Greenberg et al., 1996; Rojas et al., 1996; Williams, 1987). *Diapetimorpha introita* (Cresson), an ichneumonid ectoparasitic wasp, has also been considered as a candidate for mass release to control low density populations of *Spodoptera* sp., particularly the fall armyworm, *Spodoptera frugiperda* (Pair, 1995; Gross and Pair, 1986). *D. introita* is a native ectoparasitoid which parasitizes the pupal stage of *S. frugiperda*. After identifying the host pupation site, the adult wasp lays an egg in the vicinity of the host.

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Upon hatching, the first instar locates the host pupa and begins to feed. After approximately 5 days, the entire content of the host pupa will have been consumed (Pair, 1995).

A major deterrent to the more widespread use of *D. introita* and other wasp parasitoids is the high cost of rearing them. Currently, mass rearing requires the culture of large numbers of host insects which, in turn, are used to rear the parasitoid. The use of artificial diets to mass rear beneficials has many advantages, including reduced rearing costs (Simmonds, 1944; Grenier et al., 1994; Greany et al., 1989). An artificial diet has been developed for the parasitoid, *Catolaccus grandis* (Rojas et al., 1996) and artificial diets are in the process of being developed for *Trichogramma* sp. (Nordlund, personal communication), *Campoletis sonorensis* (Hu and Vinson, 1997a,b), *Edovum puttleri* (Hu et al., 1998) and *D. introita* (Greany and Carpenter, 1998; Carpenter and Greany, 1998). Several generations of *D. introita* have been successfully reared on artificial diet. Diet-reared wasps are as effective as host-reared wasps in searching for, parasitizing and developing on host *S. frugiperda*. However, development is slower, wasps are smaller and percent emergence and fecundity are significantly lower than for wasps reared on host pupae (Carpenter and Greany, 1998). Since differences in host species, even in closely related species, affect parasitoid quality, i.e. *Cotesia rubecula* prefers *Pieris brassicae* to *Pieris rapae* (Harvey et al., 1999), it is not surprising that artificial diets must be finely tuned for the production of high quality wasps.

More than 95% of *D. introita* reared on artificial diet reach the fifth instar and spin cocoons (unpublished results). Of these, only about 60% eclose as adults. Thus, mortality must be associated with events that occur within the cocoon during wasp metamorphosis to the adult (in the pharate pupal, pupal and pharate adult stages) and/or with adult eclosion. The current studies were undertaken to develop a system of markers to stage fifth instar *D. introita* in order to determine at which time(s) during the fifth (last) instar mortality was high, to determine if abnormalities in molting hormone titer or ecdysteroid profiles were characteristic of diet-reared *D. introita* and if so, to ascertain whether there was a correlation between stages exhibiting the highest mortality and abnormalities in ecdysteroid titers/profiles.

2. Materials and methods

2.1. Chemicals

Ecdysone (E) and 20-hydroxyecdysone (20HE) were purchased from Sigma (St. Louis, MO, USA). Twenty-six hydroxyecdysone (26HE) and 20,26-dihydroxyecdysone (20,26-diHE) were gifts from M.J. Thompson formerly of the Insect Physiology Laboratory, USDA, ARS, Beltsville, MD. Ecdysone antiserum was prepared by W.S. Bollenbacher (University of North Carolina, Chapel Hill, NC, USA) from a hemisuccinate derivative of E (at the C-22 hydroxyl group) which had been coupled to thyroglobulin. The antiserum has a high affinity for E, 20HE, makisterone A, 26HE and 20,26-diHE (Gelman and Woods, 1989). The relative affinities of E, 20HE, 20,26-diHE, and 26HE are 1.0, 0.77, 0.81, and 1.7, respectively (Jones et al., 1992; for 26HE, unpublished results). Tritiated E (63.5 Ci/mmol) was purchased from NEN Research Products (Boston, MA, USA). Grace's medium was purchased from Carolina Biological (Grand Island, NY, USA).

2.2. Insect rearing

Insects used in this study were obtained from laboratory colonies maintained at the Insect Biology and Population Management Research Laboratory, Tifton, GA, and *D. introita* were reared as described in Pair (1995) with a few modifications. Briefly, *D. introita* adults were held at 29±1°C, in a light:dark regimen of L:D 12:12 and a relative humidity of 60–80%, and were mated in Plexiglass-topped cages. Cotton soaked with distilled water provided moisture and a few drops of honey served as food for adults. *S. frugiperda* larvae were reared on meridic diet (Burton, 1969) at L:D 14:10 and at day and night temperatures of 28±1°C and 25±1°C, respectively. Two-day-old *S. frugiperda* pupae were placed in wells (1.8×0.8×0.8 cm^3) custom milled into 1.2-cm-thick Plexiglas. One *D. introita* egg was placed on each *S. frugiperda* pupa. Plastic wrap was stretched over the openings of the wells, sealing the pupae inside. Parasitized pupae were held in complete darkness at 29±1°C. After 5 days, the plastic covering was replaced with paper toweling, covered with plastic mesh canvas (2.6 count/cm) (Uniek Crafts Co., Waunakee, WI) to reduce the relative humidity within the wells. Cocoons were collected and held in petri dishes until adult emergence.

In order to collect *D. introita* eggs, *S. frugiperda* larvae were placed individually in cups containing approximately 15 ml of soil and allowed to pupate. Cups containing 2–4 day old pupae were placed in cages (for 24 h) containing *D. introita* adults. In the laboratory, under these conditions, about 100 eggs were oviposited in a single *S. frugiperda* pupal cell (Pair, 1995). Using a fine artist brush, eggs were removed from pupal cells, washed in 3% household bleach, rinsed in distilled water and placed on paper toweling to dry. Pieces of paper toweling (with eggs) were transferred to petri dishes (sealed with Parafilm to avoid desiccation) and shipped overnight to the Insect Biocontrol Laboratory (IBL) in Beltsville, MD. One-day-old *S. frugiperda* pupae were

placed individually in oval wells of a custom-designed 96-well plate and also shipped overnight to IBL.

At IBL, all insects were maintained in incubator boxes in complete darkness, at a temperature of 29±1°C and a relative humidity of 60–80%. *S. frugiperda* pupae were transferred individually to wells of a 24-well plate. Upon hatching, *D. introita* first instars were placed individually in wells that contained either artificial diet or *S. frugiperda* (2–4 day old) pupae. A moist brush was used to transfer the first instars. Upon reaching the fifth instar, parasitoids were observed daily for the next 12–14 days. To avoid desiccation, plates were wrapped in Parafilm for the first 4 days after transfer of the first instar wasps. After this time, Parafilm was no longer required.

2.3. Preparation of plates containing artificial diet

Artificial diet (containing no insect components) was prepared and packaged as described in Carpenter and Greany (1998). Twenty-four portions were packaged in sheets to fit into 24-well plates from which the bottoms had been drilled out. The drilled 24-well plate was placed over the diet sheet so that each well contained a diet dome. A rectangular piece of plastic was placed underneath the plate, the plate cover was placed on top, and the three pieces were secured with a rubber band.

2.4. Identification of developmental markers in fifth instar, pupal and pharate adult D. introita

Once the fifth instar had begun to spin a cocoon, its development could no longer be followed without slitting the cocoon and pulling back the cut ends to examine the wasp. A pair of microdissecting scissors was used to make a small hole in one end of the cocoon and then to cut longitudinally along the surface of the cocoon to the opposite end. Care was taken not to damage the developing wasp. Occasionally a wasp was punctured and had to be discarded. Wasps were examined either by pulling back the cut ends of the cocoon or by removing the individual and then returning it to the cocoon after examination. Often, cocoons spun by larvae reared on the artificial diet were imperfect and the wasp remained visible or partially visible during its entire development.

2.5. Sampling and extraction of D. introita hemolymph

Wasp larvae were anaesthetized by placing them in a petri dish containing small pieces of dry ice. The posterior tip of the wasp was cut, gentle pressure was applied and a graduated micropipet was used to collect 0.5–5.0 μl of hemolymph. For pharate adults, it was sometimes necessary to use a #0–1 dissecting pin to make a small hole in the head region from which hemolymph was collected. Hemolymph was placed in 1.5 ml microcentrifuge tubes which contained 300 μl of ice cold 75% aqueous methanol. Tubes were vortexed, and kept on ice for at least 20 min prior to centrifugation for 5 min at 4°C and 14,000***g***. Supernatants were transferred to 6 mm×50 mm borosilicate glass tubes and stored in the freezer at −10°C.

2.6. Determination of ecdysteroid titers

Tubes of size 6 mm×50 mm containing methanolic extracts were dried in a Savant Speedvac Concentrator (Forma Scientific, Marietta, OH, USA) and ecdysteroid titers were determined by radioimmunoassay (RIA) as described by Gelman et al. (1997). The radioactively labeled antigen was tritiated ecdysone (63.5 Ci/mmol). Since standard curves were generated from 20HE (50–4000 pg) hemolymph titers were expressed as pg 20HE equivalents/μl hemolymph. Student's *t*-tests ($\alpha \leq 0.05$) were used to determine if titers were significantly different for host- versus diet-reared wasps of a particular stage.

To identify times during development when titers peaked (determine whether a given ecdysteroid peak was significant), *t*-tests ($\alpha \leq 0.05$) were also used. The mean peak value was compared with the mean of the two baseline values on either side, not necessarily two adjacent points, but rather points that delineated the peak from the rest of the curve.

2.7. Determination of ecdysteroid profiles

To identify the ecdysteroids present during peak production, hemolymph extracts were subjected to reverse phase high pressure liquid chromatography (RPHPLC)–RIA as described in Gelman and Woods (1983, 1986). A Waters (Milford, MA, USA) HPLC system fitted with a Waters 484 tunable detector (λ=254 μm) was used. For selected wasp stages, hemolymph was collected and extracted as described above. Extracts were combined as necessary so that each sample contained at least 5000 pg of ecdysteroid. Extracts were dried, brought up in 50 μl of 40% aqueous methanol and injected onto a Waters RPHPLC μBondapak-C_{18} column (3.9 mm×30 cm; 10 μm particle size). The solvent was 40% aqueous methanol, the flow rate was 1.0 ml/min and the temperature was 32°C. Fractions of 0.6 ml were collected in 6 mm×50 mm tubes and stored in the freezer at −10°C. Prior to RIA, tubes were vacuum dried.

When calculating the concentration of ecdysteroid in each fraction, it was necessary to correct for percent recovery which was typically 60–85%. Identification of ecdysteroids was based on retention times which were compared with those of authentic standards. To confirm the identity of E, 20HE and 20,26-HE, sufficiently concentrated samples were run on RPHPLC, aliquots were

taken for RIA and the remainder of RPHPLC fractions whose elution times were the same as a given standard were pooled, dried, reconstituted in normal phase solvent and injected individually onto a normal-phase Shandon hypersil silica gel column (4.6 mm×25 cm; 5 μm particle size). The eluting solvent was 125 parts methylene chloride, 35 parts isopropanol and 2.5 parts water, the flow rate was 1.0 ml/min and the temperature was 22–24°C. Fractions of 1.0 ml were collected in 3 ml vials and aliquots were taken for ecdysteroid RIA. Again, elution times were compared with those of known standards.

3. Results

3.1. Developmental timetable for D. introita fifth instars, pupae and pharate adults

Markers selected to track the developmental progress of *D. introita* from the beginning of the fifth instar through adult eclosion are listed in Table 1. Wasps reared on the artificial diet developed more slowly than those reared on their natural host pupae. Host-reared *D. introita* typically began to spin on day 5 and the eye began to enlarge and move backwards on day 7. Diet-reared wasps did not first exhibit these characteristics until day 7 and 10, respectively. The development of the pharate pupa (enlargement of the eye, crinkling of the cuticle) and gut purge also occurred at a slower rate in the diet-reared wasps. In addition, diet-reared *D. introita* exarate pupae remained white for 3 days, compared with host-reared exarate pupa which only remained white for 2 days. Once the pharate adult cuticle had begun to darken, development of the diet- and host-reared wasps occurred at approximately the same rate, typically taking 4 days. Eclosion was observed the day after the abdomen had become dark brown (♀) or exhibited dark black stripes (♂). To verify that slitting the cocoon did not slow wasp development, some cocoons were left intact, and wasps were removed and examined at specific times after spinning had begun. For both the diet- and host-reared larvae, developmental progress was similar whether or not the cocoon had been slit.

3.2. Hemolymph ecdysteroid titers in host- and diet-reared D. introita

In order to increase synchrony when determining hemolymph ecdysteroid titers, the stages listed in Table 1 were divided into two or more substages (Fig. 1). In both host- and diet-reared wasps that had finished feeding, but had not yet begun to spin, hemolymph titers were very low. Titers remained low during spinning, and began to increase as the eye enlarged and moved backward out of the head into the prothorax. This backward movement of the eye is associated with head capsule slippage. Apolysis has occurred, new cuticle formation has begun, and although the new cuticle is not completely formed, the pharate pupa has begun to slip backwards. As the wasp proceeded through Eye 1, Eye 2 and Eye 3 stages, the eye became larger, 0.04–0.08 mm, 0.1–0.15 mm and 0.3–0.4 mm in width, respectively. Typically, at the Eye 1 stage, the eye is located in the back of the head or at the junction of the head and the prothorax. At the Eye 2 stage, the eye is located in the front half of the prothorax and at the Eye 3 stage, the eye is in the back half of the prothorax. During this enlargement and backward migration of the eye, ecdysteroid titers increased. However, it was only in the Eye 1 and Eye 2 stages that hemolymph ecdysteroid titers in host-reared wasps were significantly higher than those in diet-reared wasps. Titers continued to increase during early

Table 1
Developmental timetable for *D. introita* fifth instars, pupae, and pharate adults[a]

Stage	Days post-hatch[b]	
	S. frugiperda	Artificial diet
Spinning	5	7
Eye begins to enlarge	7	10
Cuticle is crinkled	8	11
Gut purge	8	12
Exarate pupa		
White (eye reddish brown)	9	13
White (eye black)	10	14
Thorax dark or head and thorax dark	11	16
Abdomen		
(brown ♀)	12	17
(striped ♂)		
Adult eclosion	14	19

[a] Temperature 29±1°C; relative humidity 60–80%.
[b] Day at which specified stage was first observed.

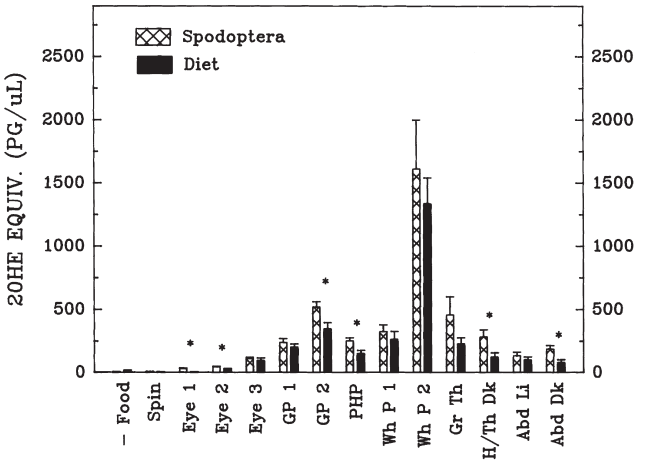

Fig. 1. Hemolymph ecdysteroid titers in host- and diet-reared *D. introita*. Hemolymph was extracted in aqueous methanol and subjected to ecdysteroid RIA as described in Section 2. Titers are expressed as 20HE equivalents per µl of hemolymph. Each bar represents the mean±SE of at least ten separate determinations. Standard errors not shown were smaller than the size of the datum point. For starred (*) stages, hemolymph ecdysteroid levels were significantly higher in host-reared compared with diet-reared parasitoids. Food= wasps that have stopped feeding; Spin=wasps that have begun to spin a cocoon; Eye 1=eye that is 0.04-0.08 mm and is located in the back of the head or at the junction of the head and the prothorax; Eye 2=eye that is 0.1-0.15 mm and is located in the front half of the prothorax; Eye 3=eye that is 0.3-0.4 mm and is located in the back half of the prothorax; GP 1=early gut purge; GP 2=late gut purge (gut purge is almost completed); PHP=pharate pupae that remained intact after manual removal of the old larval cuticle; Wh P 1=day-1 exarate pupae; Wh P 2=day-2 exarate pupae; Gr Th=thorax that has begun to darken; H/Th Dk=head and thorax that are black; Abd Li=abdomen that is either light brown (♀) or has light grey stripes (♂); Abd Dk=abdomen that is either dark brown (♀) or has black stripes (♂).

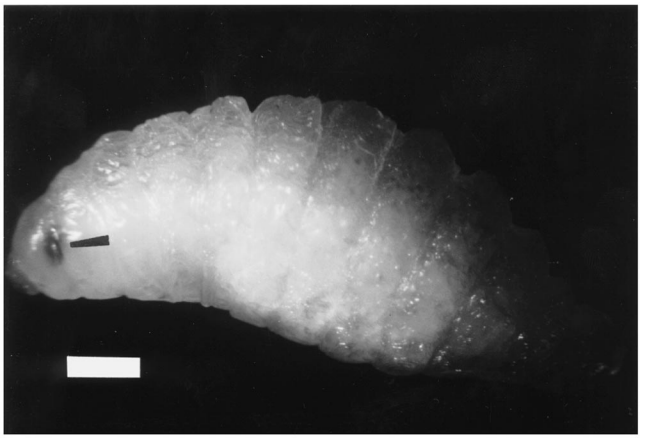

Fig. 2. *D. introita* pharate pupa in the process of gut purge (GP 1). The wasp was removed from the cocoon as described in Section 2. The eye (tip of pointer) has migrated to the back of the prothorax and the cuticle is crinkled. The length of the bar in the lower left hand corner is 1.2 mm.

gut purge (GP 1, Fig. 2), and peaked as gut purge was almost completed (GP 2). In host-reared GP 2 wasps, titers were significantly higher than in diet-reared wasps. For the wasp shown in Fig. 2, the eye is at the very back of the prothorax, and the cuticle is quite crinkled. According to Hinton (1946), the onset of the pharate stage is associated with apolysis and the designation continues until eclosion is initiated. Crinkling of the old larval cuticle was first observed in wasps in the Eye 3 stage, and pupal legs first became apparent in individuals which were in the process of early gut purge. In our studies the term 'pharate pupa' (PHP) was assigned to pharate individuals in which the larval cuticle could be peeled off and the pupae remained intact. In PHP, ecdysteroid titers decreased, but levels remained significantly higher in host-reared larvae. With the formation of the exarate pupa (eye is reddish brown in the day-1 exarate pupa), ecdysteroid titers again began to increase and peaked in day-2 exarate pupae (eye is black). In these day-2 exarate pupae, although the pupal cuticle could be peeled off, the new pharate adult cuticle was very thin and fragile, especially in the posterior portion of the wasp. As the thorax began to darken (typically observed before the head began to blacken), titers decreased sharply and continued to decrease/level off through the remainder of adult development. Importantly, in the 'dark head and thorax' and 'dark abdomen' stages, hemolymph ecdysteroid titers were significantly higher in the host-reared than in the diet-reared wasps, and were higher, but not significantly so, in host-reared wasps at the 'grey thorax' stage. At all stages sampled, there was no significant difference in hemolymph ecdysteroid titers of male and female wasps (results not shown).

3.3. Determination of percent mortality for each stage

For those individuals that died prior to adult eclosion, the stage at which death occurred (development ceased) was noted. Of the total number of diet-reared wasps (n=101 for this experiment) that died before adult eclosion was completed (approximately 40%), the highest rate of mortality (42%) occurred in those pharate adults in which the abdomen was dark (Fig. 3). Another 42% of the 40% died either just after the eye had begun to migrate backwards (Eye 1) or in PHP, 21% for each of these stages. Finally, approximately 8.0% died just after the abdomen had begun to turn brown (Abd Li), and the remaining 8.0% died during adult eclosion (Ecl Adult). Only 15% mortality (n=59) was observed in host-reared *D. introita*. Mortality was distributed approximately equally among Eye 3, GP 2, PHP, Abd Dk and Ecl Adult stages. Mortality was lowest in the PHP stage (results not shown).

3.4. Ecdysteroid profiles for D. introita hemolymph

HPLC-RIA of methanolic extracts of hemolymph was used to generate profiles for samples collected from wasps that had just completed gut purge (Fig. 4) and

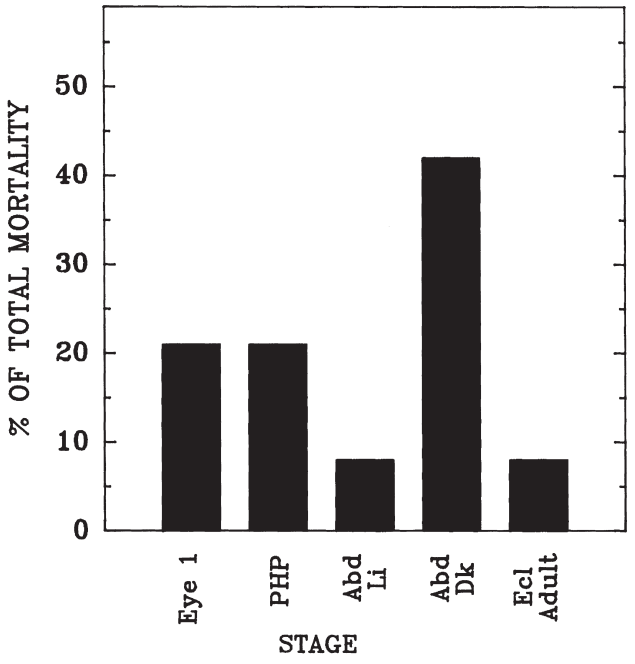

Fig. 3. Percent mortality for selected stages of diet-reared *D. introita*. The stage at which death occurred=that stage at which development ceased. Results are expressed as a percentage of the total number of wasps (40% of 101 wasps=40 wasps) that died. Ecl Adult=adult in the process of eclosion; for definitions of other abbreviations, refer to the legend for Fig. 1.

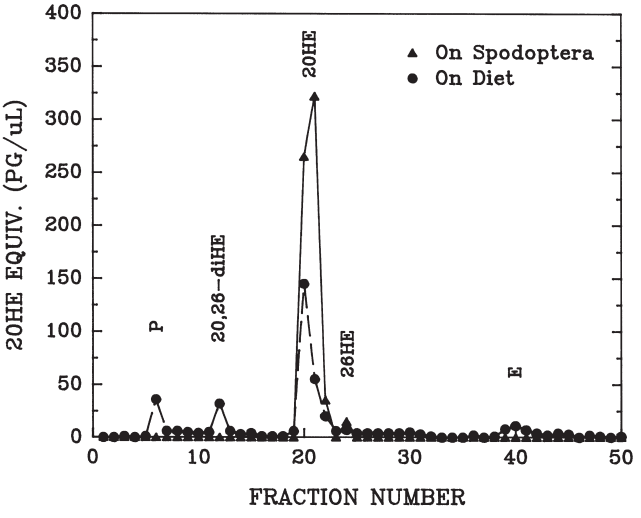

Fig. 4. Hemolymph ecdysteroid profile for *D. introita* in which gut purge has almost been completed (GP 2). Hemolymph was collected and extracted and subjected to RPHPLC-RIA as described in Section 2. Results are from a single run and are expressed as pg 20HE equivalents per µl hemolymph. Duplicate experiments generated similar profiles. The identities of E, 20HE and 20,26-diHE were verified by normal phase HPLC-RIA (see Section 2). 26HE=26-hydroxyecdysone; P=polar ecdysteroids.

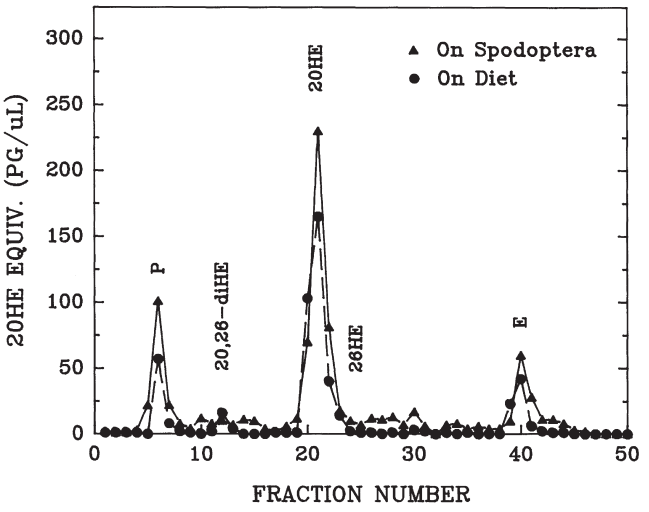

Fig. 5. Hemolymph ecdysteroid profile for *D. introita* day-1 exarate pupae (Wh P 1). For explanation, refer to the legend for Fig. 4.

from day-1 and day-2 exarate pupae (Figs. 5 and 6, respectively). For each stage, results are from a single HPLC run. These profiles and those from duplicate runs revealed that E and 20HE were the major ecdysteroids present in hemolymph from both host-reared and diet-reared wasps. A relatively large peak containing polar ecdysteroids was also present in hemolymph from day-1 exarate pupae. 26HE and 20,26-diHE, if present, occurred at low/very low levels. A small peak of 20,26-diHE was observed in hemolymph extracts prepared from diet-reared GP 2 and diet-reared day-2 exarate pupae. Since the ecdysteroid with the same retention time as 26HE (has a very high affinitiy of the antiserum), when present, always occurred at very low levels, its identity was not verified by normal phase HPLC.

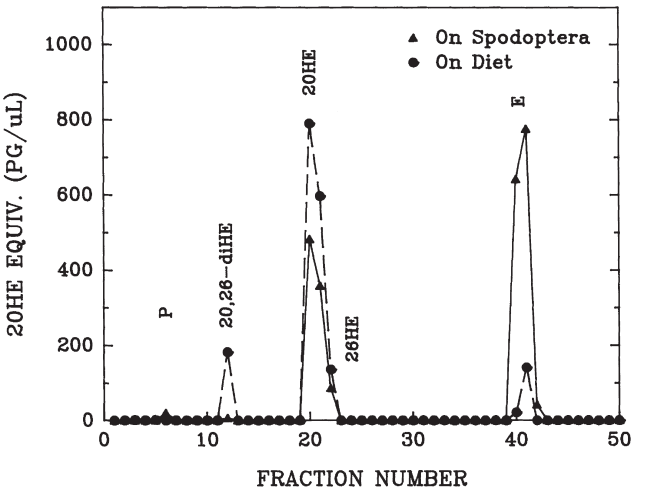

Fig. 6. Hemolymph ecdysteroid profile for *D. introita* day-2 exarate pupae (Wh P 2). For explanation, refer to the legend for Fig. 4.

4. Discussion

A system of markers has been devised to track the development of *D. introita* from the time cocoon spinning began (late in the fifth instar stage) through adult eclosion. Although as reported by Carpenter and Greany (1998), wasps reared on an artificial diet develop more slowly than wasps reared on the natural host (Table 1), both diet- and host-reared *D. introita* passed through the same stages of development. In each, in preparation for pupation, the eye enlarged and moved posteriorly, the cuticle became crinkled, gut purge ensued and upon ecdysis, the exarate pupa emerged. The pattern of darkening in the exarate pupa, thorax followed by head and then abdomen, was also similar in both diet- and host-reared wasps (Table 1). Although the time of apolysis (separation of the old cuticle from the epidermis) was not pinpointed using histological techniques, it was apparent that in fifth instars, apolysis must have occurred prior to the initiation of eye movement, since the backward motion of the eye is associated with pharate pupal withdrawal from the old head capsule. Apolysis and new cuticle formation, which has been reported to proceed along an anterior to posterior gradient (Rembold et al., 1980; Riddiford, 1985), followed this pattern in *D. introita*. When attempts were made to peel away the larval cuticle in wasps undergoing gut purge, the anterior portion (head and thorax) of the pharate pupa was much more developed than the posterior portion (abdomen). Cuticle crinkling (probably indicative of the deterioration of the old fifth instar larval cuticle just prior to ecdysis) was first observed at the Eye 3 stage, and pupal legs were observed in pharate individuals as early as GP 1. The degree of cuticle crinkling increased during gut purge and soon after gut purge was completed, a fully formed pharate individual could be found upon removal of the old larval cuticle. It is interesting that in these wasps and probably in Hymenoptera in general, apolysis and new cuticle development occurred/occur before gut purge. In Lepidoptera, gut purge precedes the initiation of molting (apolysis and new cuticle formation) (Bollenbacher et al., 1975; Gelman and Hayes, 1982; Riddiford, 1985; Nijhout, 1994). Also of significance is the observation that head capsule slippage, as evidenced by the backward migration of the eye, began very early in *D. introita* pharate pupal formation. It was first observed when the new pupal cuticle did not have structural integrity in either the anterior or posterior portion of the wasp.

The rise and fall of hemolymph ecdysteroid levels from the time of cocooning until adult eclosion was as expected in both diet- and host-reared *D. introita* with hormone levels increasing before each molt and decreasing after the formation of the new pharate (pupal or adult) individual (Fig. 1). In other hymenopterans (the honey bee, *Apis mellifera* and the bumblebees, *Bombus hypnorum* and *B. terrestris*), fluctuations in hemolymph ecdysteroid levels during the larval pupal transformation were similar to those observed for *D. introita* (Strambi et al., 1984; Rachinsky et al., 1990). As *D. introita* approached apolysis, and just after apolysis when the eye had begun to enlarge and migrate backwards, ecdysteroid levels, although they began to increase, were surprisingly low. In diet- and host-reared wasps, these titers were 3 pg/μl and 31 pg/μl, respectively (Fig. 1). It was not until the Eye 3 stage that titers reached approximately 100 pg/μl, a value that is the same as that reported for the endoparasitoid *Pimpla instigator*, just prior to ecdysis to the pupa (Claret et al., 1978). Titers peaked in *D. introita* that were completing gut purge (347 pg/μl and 520 pg/μl, respectively, in diet- and host-reared wasps). In *Bombyx mori* and other lepidopterous insects, a very low concentration of ecdysteroid is sufficient to induce spiracle apolysis and/or other localized apolysis, but ecdysteroid titers typically must be at near peak levels to induce apolysis of the body epidermis. Epicuticle deposition begins slightly later, and the rest of the cuticular layers are deposited as titers decrease (Kiguchi and Agui, 1981; Riddiford, 1985). Also, as mentioned previously, while *D. introita* purges its gut after apolysis and the initiation of pupal cuticle formation, gut purge in lepidopteran larvae precedes the premolt ecdysteroid peak and the accompanying apolysis and new cuticle production. In the beetle *Tenebrio molitor*, apolysis and secretion of the ecdysial membrane begin prior to gut purge, and prior even to the small rise in ecdysteroid (commitment peak) that occurs in the last instar (Delbecque et al., 1978). Riddiford (1985) suggests that for *T. molitor*, a decline in juvenile hormone (JH) titers may be responsible for inducing apolysis. Whether or not fluctuations in JH levels are involved in triggering apolysis in *D. introita* is not known. In bumble bee prepupae, fluctuations in juvenile hormone and ecdysteroid levels paralleled each other (Strambi et al., 1984).

RPHPLC-RIA indicated that for both diet- and host-reared wasps in the GP 2 stage, almost all of the ecdysteroid present was in the form of physiologically active 20HE (Fig. 4). This was also true for GP 1 individuals (results not shown). Thus, it appears that 20HE serves to regulate physiological events which occur during gut purge and maturation of the pharate pupa and perhaps, to trigger apolysis and the initiation of new cuticle synthesis. The second ecdysteroid peak was observed in day-2 exarate pupae (Fig. 1). Apolysis and pharate adult formation must have occurred sometime between day 1 and day 2 post-pupation, since in day-2 exarate pupae, the adult cuticle was only in the early stages of formation. In both day-1 and day-2 exarate pupae, major peaks of 20HE and its immediate precursor, E (Koolman, 1982; Beydon et al., 1981), were observed (Figs. 5 and 6). In day-2 exarate pupae, levels of E are much higher

in host-reared than in diet-reared *D. introita* (Fig. 6). This difference may be due to the slower rate of development exhibited by diet-reared wasps. In the latter, the thorax typically does not darken until day 4, while in host-fed wasps, the thorax begins to darken on day 3. No makisterone A was detected in hemolymph subjected to RPHPLC-RIA (Figs. 4–6). In contrast, in the honey bee, *A. mellifera*, the major ecdysteroid present in pupal hemolymph was makisterone A (Feldlaufer et al., 1985). Both makisterone A and 20HE were present in prepupal queen hemolymph (Rachinsky et al., 1990). The presence of makisterone A is associated with the inability of the insect to convert C_{28} and C_{29} sterols to cholesterol and the absence of a constant supply of cholesterol in the insect's diet (Feldlaufer et al., 1985). Since *S. frugiperda* is the food source for *D. introita*, the wasp's diet should be rich in cholesterol, and therefore, it was expected that 20HE rather than makisterone A would be the major ecdysteroid observed. In last instars of *Cotesia congregata* (Hymenoptera: Braconidae), an endoparasitoid of *Manduca sexta*, 20HE rather than makisterone A was also the molting hormone. E, 20,26-diHE, 26HE and a peak containing polar ecdysteroids were also present in *C. congregata* hemolymph sampled the day before parasitoid emergence and its concomitant molt to the third (last) instar (Gelman et al., 1998). A relatively large peak containing polar ecdysteroid was present in day-1 *D. introita* exarate pupal hemolymph, but surprisingly, not in hemolymph from day-2 exarate pupae. Since polar ecdysteroids are inactivation products of physiologically active ecdysteroid (Beydon et al., 1981), we expected that the level of polar ecdysteroids would increase after the initiation of pharate adult development which was well underway in day-2 exarate pupae.

Of major importance are the significant differences observed in hemolymph ecdysteroid levels in diet- and host-reared wasps at key times in development (Fig. 1). These occurred at the Eye 1, Eye 2, GP2, PHP, H/Th Dk and Abd Dk stages. Interestingly, of the total mortality observed (40% for diet-reared wasps), mortality was especially high in Eye 1, PHP and Abd Dk individuals (Fig. 3). Some mortality was also observed in Abd Light wasps (the stage between H/Th Dk and Abd Dk wasps) and in adults in the process of eclosing (the stage immediately following the Abd Dk stage). At first, one might think it surprising that a lower ecdysteroid level is correlated with relatively high percentages of mortality in PHP, Abd Dk and Ecl Adult stages, since ecdysteroid titers must drop (reported for lepidopterans) in order for eclosion hormone to be released (Truman, 1985; Horodyski, 1996). However, the level to which titers must drop in Hymenoptera is not known, and certainly, in Abd Dk wasps, hemolymph ecdysteroid titers are quite low in comparison with those observed for Wh P 2 wasps, exarate pupae in which hemolymph ecdysteroid titers peaked (Fig. 1). Overall, mortality in host-reared wasps was only 15%. Five stages exhibited approximately equal mortality of which three, PHP, Abd Dk and Ecl Adult, were the same as those that exhibited high mortality when reared on artificial diet. These stages, then, appear to be the most fragile and in diet-reared wasps, their vulnerability is magnified.

Since the occurrence of abnormally low hemolymph ecdysteroid levels in diet-reared wasps is correlated with high percentages of developmental arrest and ultimately, mortality within the cocoon, it might prove beneficial to add ecdysteroid to the artificial diet. (Cholesterol levels in the diet are already high, since the two major components of the diet are beef liver and egg yolk.) However, since hemolymph ecdysteroid levels fluctuate during wasp development, the effects of adding various concentrations to the diet must be monitored carefully. Ecdysteroid titers should be relatively high in day 2–4 host pupae which are preferred by newly hatched *D. introita.* Therefore, wasps may secure some of their required ecdysteroid directly from their host. It is also possible that *D. introita* does not depend upon the host for ecdysteroid, but rather, synthesizes all of its ecdysteroid from cholesterol. If the latter scenario is correct, the increased mortality observed in diet-reared wasps may be due to the presence or absence of some other dietary factor which indirectly affects the wasp's ability to synthesize ecdysteroid. Thus, Campbell and Duffey (1979, 1981) have reported that the endoparasitoid *Hyposoter exiguae* exhibits molting and structural abnormalities when reared on hosts fed on artificial diets containing α-tomatine, a compound obtained from tomato plant extracts.

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